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Retinal phospholipase C from squid is a regulator of Gqa GTPase activity

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Abstract

The phospholipase C (PLC) pathway is the major signaling mechanism of photoactivation in invertebrate photoreceptors. Here we report the cloning of a cDNA encoding a 140-kDa retinal PLC that is uniquely expressed in squid photoreceptors. This cDNA encodes a protein with multiple distinct modular domains: PH, X and Y catalytic, and C2 domains, as well as G- and P-box motifs and two GTP/ATP binding motifs The PLC was simulated by activated squid Gqa but not by squid Gqbg or mammalian bg subunits. The PLC was inhibited by monophosphate, diphosphate and triphosphate nucleotides but not cyclic nucleosides. We also tested the

the rhabdomeric membranes. Depletion of PLC-140 from the rhabdomeric membranes decreased the GTP hydrolysis but not GTPgS binding to the membranes. Reconstitution of puriæd PLC-140 with membranes accelerated Gqa GTPase activity by &vefold at a concentration of 2.5 m suggest that PLC-140 plays an important role in both the activation and inactivation pathways of invertebrate visual transduction.

Keywords: Gqa, GTPase, phospholipase C, photoreceptor,

squid.

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ability of PLC-140 to regulate the GTPase activity of Gga in

The phosphoinositide-speci®c phospholipase C (PLC) enzymes are a family of proteins that hydrolyze membrane phosphoinositol-4,5 bisphosphate, to generate the second messengers inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). PLC proteins are found in both vertebrate and invertebrate species as well as in yeast, slime molds,

species including: Musca (DeVary et al. 1987), Limu (Fein 1986), and squid (Mitchell et al. 1995; Suzuki e 1995). Several invertebrate PLCs have been cloned a include two PLC isoforms from Drosophila melanoga PLC-norpA and PLC-p21 (Bloomquist et al. 1988; Shortridge et al. 1991), and one from lobster (Xu and

fungi and plants (Rebecchi and Pentyala 2000). Thus far, 11 mammalian PLCs have been identi®ed and they have been classi®ed into four distinct groups: PLC-b, PLC-g, PLC-d and PLC-1. Of these four classes, the PLC-b and PLC-1 isozymes demonstrate G protein-regulated activity. The PLC-b type enzymes are regulated by G protein a subunits of the Gq family (Kozasa et al. 1993; Lee and Rhee 1995; Biddlecome et al. 1996), as well as by G protein bg subunits (Waldo et al. 1991; Park et al. 1993a; Wu et al. 1993b) while PLC-1 is regulated by monomeric Ras (Kelley et al. 2001)

The PLC pathway is the major signaling pathway in invertebrate photoreceptors. The molecular mechanism entails the photoactivation of rhodopsin leading to the stimulation of invertebrate Gq, which in turn activates retinal PLC. The role of G protein-regulated PLC activity in vision has been studied most extensively in photoreceptors of Drosophila (Bloomquist et al. 1988; Running Deer et al. 1995), but have also been reported in other invertebrate

McClintock 1999). A cDNA encoding a truncated PL enzyme was also isolated from squid (Carne et al. 199 We have previously reported the isolation and puri®c a 140-kDa PLC protein from squid photoreceptors an shown it to be regulated by Gqa (Mitchell et al. 1995 we report the cloning of the full-length cDNA encodi 140-kDa PLC protein and demonstrate its regulation protein subunits and nucleotides as well as its ability regulate the GTPase activity of invertebrate Gqa.

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Abbreviations used: DAG, diacylglycerol; IP3, inositol-1,4,5-trisphosphate; PLC, phospholipase C.

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Materials and methods

Cloning of squid phospholipase C Oligonucleotide primers were designed based on previously published peptide sequences derived from a puri®ed 140-kDa PLC protein (Mitchell et al. 1995). PCR reactions were performed using a squid retinal cDNA library as template (a generous gift from Drs J. K. Northup and M. Brownstein of NIH, Bethesda, MD, USA). A 472 base pair PCR product was generated that showed high sequence similarity to a truncated squid PLC (Carne et al. 1995) and primers based on the sequence from this truncated PLC produced a fragment of approximately 2600 base pairs. Further sequence information on the 3 H end of the PLC cDNA was obtained using primers based on peptide four from puri@ed PLC-140, and RACE (Sambrook and Russell 2001) reactions were performed to H and 3 H UTRs using the gene-speci®c primers GCCTTAAAATCAAACAAAGCFTATACCAAAATTAT; TCC-AGTACAAGAAATATCCCTTGGGATCAA and the vectorspeci®c primers TAATACGACTCACTATAGGGAGACCGGAAG; AAGGTTCCTTCACAAAGATCCTCTAGAGTC. Nucleotide sequ-

ences were determined for both strands of several positive clones

nitrocellulose membranes and immunoblotted with a polyclonal antibody raised against puri@ed endogenous squid PLC-140 (Towbin et al. 1979).

GTPgS binding, GTPase and phospholipase C assays
GTPgS binding to rhabdomeric membrane preparations was

from each PCR reaction by automated DNA sequencing at a local facility. The full-length sequence obtained (GenBank accession number AF258528) was compared with DNA sequences in GenBank. Simple Modular Architecture Research Tool (SMART) was used to determine the domain topology of the protein encoded by the cDNA (Schultz et al. 2000) and PROSITE database search (Hofmann et al. 1999) was used to identify biologically signi@cant sites encoded by the cDNA. PEST @nd program (Rogers et al. 1986; Rechsteiner and Rogers 1996) was used to identify PEST sequences in PLC-140.

Expression of recombinant PLC-140 in Escherichia coli

The protein encoded by the PLC-140 cDNA was expressed in

BL21-SI cells using Gibco BRL Gateway Cloning Technology

(Gibco-BRL, Gaithersburg, MD, USA). The full-length coding

region of PLC-140 cDNA was PCR ampli@ed using Clontech

Advantage HF-2 PCR kit (Clontech Laboratories, Palo Alto, CA,

USA) with attB1-FS and attB2-FA primers. Entry clones were

generated in DH5a cells using the full-length attB PCR product and
attP containing pDONR201 vector. Plasmid DNA from positive

entry clones were isolated and used to generate His-tagged

expression clones in pDEST17 vectors.

Bacterial BL21-SI colonies expressing His-tagged PLC-140 were induced with 0.3 m NaCl. PLC-140 protein expression was analyzed via western blotting using antiserum raised against puri®ed PLC-140. Recombinant PLC-140 (rPLC-140) protein was puri®ed from bacterial extracts under denaturing conditions (8.0 m PO , 500 mm NaCl) with urea, 10 mm Tris pH 8.0, 100 mm NaH Ni-NTA resin (Qiagen, Valencia, CA, USA) with slight modi®cations to manufacturer's instructions. The resin was washed in buffer containing 6 m urea, 20 mm Tris pH 8.0, 500 mm NaCl, 50 mm imidazole, and the proteins renatured using a stepwise gradient from 6 to 0 m urea in 20 mm Tris pH 8.0, 500 mm NaCl in 0.5 m increments prior to elution. The semipuri@ed recombinant PLC protein (PLC was assessed as approximately 50% of the protein content of eluted fractions) was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS±PAGE) on 8% polyacrylamide gels (Laemmli 1970), transferred to

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determined by incubating 5 mg of membranes in 20 mm NaHEPES pH 7.5, 3 mm MgCl , , 200 nm GTPgS (50 000 cpm/pmol) at 2 At times indicated in individual experiments, binding reactions were stopped by dilution with 2 mL of ice-cold stop buffer (20 mm NaHEPES pH 7.5, 3 mm MgCl ,) followed by rapid ©l nitrocellulose ©lters. After washing ©lters three times in 3 mL of stop buffer the ©lters were dried and [38 S]GTP liquid scintillation counter.

GTPase activity was assessed in rhabdomeric membranes under conditions in which the GTP concentration is lower than that of Gqa by incubating 10 mg of membranes (containing 1.2 pmole Gqa) at 228C in 20 mm NaHEPES pH 7.5, 3 mm MgCl [g- 37 P]GTP (50 000 cpm/pmol). GTP hydrolysis was initiated by the addition of membranes and puri®ed endogenous PLC and stopped by addition of 100 mL of 6% perchloric acid. Nucleotides were removed by addition of 700 mL of 10% activated charcoal in 20 mm NaPO buffer pH 7.5 and free [measured by scintillation counting.

Regulation of puri@ed endogenous PLC-140 activity was measured by reconstituting aliquots of Gqa (1 ng), Gqbg (10 ng) or Gtbg (175 ng) with PLC-140 (2 ng) in the presence of [PIP _ ,/PE vesicles in the presence of 1 mm free Ca volume of 50 mL as described previously (Mitchell et al. 1995; Bamsey et al. 2000).

Data presentation

Means of triplicate values obtained from at least two experiments are reported ^SD, unless otherwise noted in the @gure legends.

Results

Amino acid sequence, domain structure and tissue distribution of squid phospholipase C (PLC-140)

The full-length cDNA encoding squid retinal PLC-140 had a 3381 nucleotide open reading frame. The deduced amino acid sequence derived from this cDNA is shown in Fig. 1(a) along with the sequence of the two other PLC isozymes

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expressed in retinal photoreceptors; mammalian PLC-b4 and Drosophila norpA. The four peptides derived from puri®ed PLC-140 on which the oligonucleotide primers were based were all present within the cDNA. When transformed into E. coli the cDNA encoding PLC-140 produced a protein that was similar in size to the native PLC found in squid membranes as well as the PLC puri®ed from these membranes. The recombinant protein was

Fig. 3 Tissue distribution of PLC-140. Homogenates prepared from various squid tissues (10 mg for retina and 30 mg for all other tissues) were subjected to SDS PAGE followed by western blot analysis with an antibody raised against puri®ed PLC-140. Dashes on the right side of the &gure indicate the migration of molecular weight standards from top: a 2, -macroglobulin (180 000), b-galactosida (116 000), fiructose-6-phosphate kinase (84 000), pyruvate kinase (58 000), fumarase (48 500), and lactic dehydrogenase (36 500).

similarity was found as anticipated to the truncated northern European squid PLC (Carne et al. 1995). PLC-140 also had signi®cant similarity with the two Drosophila PLCs: norpA and PLC-21 (37%), and a recently identi®ed PLC from lobster (35%) (Xu and McClintock 1999). Identity of PLC-140 with the mammalian PLC isozymes of the b family ranged between 39 and 40% with PLC-b1 and b4, and 36±39% identity with PLC-b2 and b3.

The domain structure of PLC-140 as determined using SMART module was found to include six distinct domains: pleckstrin homology (PH), X and Y catalytic, C2, G- and P-boxes. There were also two ATP/GTP-binding site motifs

recognized by an antibody raised against puri®ed PLC-140 as demonstrated by western blot analysis (Fig. 2).

Comparison of the deduced amino acid sequence of this PLC with other sequences in the database revealed the protein to be similar in structure and organization to other members of the PLC-b family of proteins. Highest sequence

Fig. 1 (a) Alignment of the deduced amino acid sequence of squid PLC-140 (AF258528) with Drosophila norpa PLC (norpa: AF181641) and rat PLC-b4 (A48047) The asterisks denote conserved amino acid residues in all the aligned sequences, the dots denote residues that are conserved in two or more proteins and the double dots denote conservative amino acid substitutions. The dashes represent gaps introduced for optimal alignment. (b) Domain structure of

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identi®ed within the PLC sequence (Fig. 1b).

A survey of tissues taken from Loligo pealei by immunoblot using a polyclonal antibody raised against puri®ed PLC-140 demonstrated expression of this protein only in the eye (Fig. 3). Neither the peripheral tissues nor the optic ganglion demonstrated any immune reactivity to

PLC-140. It contains six distinct modular domains: pleckstrin homology domain (PH: 22±144), PLC catalytic domains (X: 323±471 and Y: 514±630) and calcium binding domain (C2: 651±750), P- and G-box motifs span amino acids 1016±1047 and 1069±1096, respectively. A putative PEST sequence region (score 1 3.26) designates the putative site of calpain cleavage (858±881) and designates ATP/GTP binding site motifs (344±351 and 815±822).

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Table 1 1 "SIGTPeS hinding to puri@ed PLC.140	Table 1	35	SIGTPeS binding to puri@ed PLC-140
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Additions	[³⁰ S]GTPgS
None	100 ^ 9
GMP i m M	18 ^ 3
GDP 1 m 🙀	34 ^ 12
GTP 1 m M	25 ^ 4
GTPgS 1 m M	19 ^ 5
cGMP l m M	137 ^ 13
ATP 1 m	43 ^ 12
cAMP I m M	128 ^ 15
Mg ²¹ 10 m _M	99 ^ 4
Ca ²¹ 1 m _M	123 ^ 14

A total of 2 ng of puri@ed PLC-140 were incubated with 120,000 cpm

[33 S]GTPgS (923,000 cpm/pmol) for 30 min in the presence of the indicated compounds. Samples were quenched and [

protein determined as described in Materials and methods. Results are shown as percentage of [

33 S] bound to PLC-140 additional compounds (4.3 ^ 0.4 finoles GTPgS bound) and are the mean ^ SD of triplicate determinations.

activity approximately 70% over basal activity. Addition the same concentration of Gqa activated by AlF resulted

Fig. 4 Regulation of PLC-140 activity by G proteins and ribonucleotides. (a) Puri@ed endogenous PLC-140 (2 ng) was incubated with phospholipid vesicles containing PIP2 in the absence (control) or M NaF, 2 m M AlCI 3 (AlF control), 1 ng presence of 1 ng of Gqa, 1 m), 10 ng of Gqbg puri®ed from squid of activated Gq (Gqa-AlF rhabdomeric membranes, or 175 ng of Gtbg puri®ed from bovine ROS. (b) Puri&ed PLC-140 was assessed in the absence (control) or presence of the indicated compounds at a @nal concentration of 0.1 M as described under Materials and methods. The results are means ^ SD of two experiments and are expressed as a percentage of the activity in the absence of G protein subunits or nucleotides. The activity in control samples corresponding to 100% was $15 \ ^{\circ} 2$ pmol/min and $17 \ ^{\circ} 3$ pmol/min in experiments a and b, respectively.

the PLC antibody, indicating that the expression of this PLC protein is very speci®c to the squid photoreceptors.

Regulation of PLC-140 by Gqa, Gbg and nucleotides

The similarity in structure between PLC-140 and the other retinal PLC-b isozymes, norpA and PLC-b4, suggested that squid retinal PLC may show a similar pattern of regulation as these other proteins. We tested the effect of both squid and mammalian visual Gbg on endogenous puri®ed

PLC-140 in reconstitution assays and compared this to the activation seen by Gqa subunits that we have reported previously. Addition of 0.45 nm Gqa stimulated the PLC

increased activation of PLC-140 to 10-fold stimulation the basal activity of PLC-140 in the presence of AIF alo (Fig. 4a). Addition of a molar excess of either squid 6 n Gbg or 100 nm mammalian retinal Gb the PLC activity. The presence of GTP/ATP binding m in the PLC-140 sequence suggested that nucleotides ma regulate its activity and this was demonstrated for both guanine and adenine nucleotides that were both found t inhibit PLC activity in a concentration-dependent mann (Fig. 4b). PLC-140 was able to bind [stoichiometry of approximately 0.3 mole of GTPgS/mo PLC and this binding was inhibited by unlabeled guanin monophosphate, guanine diphosphate and guanine triph phate as well as adenosine triphosphate; however, cycli adenosine monophosphate or cyclic guanine monophos were not able to displace GTPgS. Binding of nucleotide PLC-140 was unaffected by the presence of magnesium was slightly enhanced in the presence of calcium (Table

PLC regulation of Gqa GTPase activity
PLC-bl has previously been demonstrated to increase t rate of GTP hydrolysis of mammalian Gqa (Chidiac and Ross 1999). Given the similarity between the squid Gqa protein and its mammalian counterparts of the Gq famil (Ryba et al. 1993) we decided to test the effect of PLC-on the GTPase activity of squid Gqa. The squid Gq pro binds GTP very rapidly in the presence of light activate rhodopsin but extremely slowly when removed from th rhabdomeric membranes, with rate constants for GTPgS

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Fig. 5 [38 S]GTPgS binding and GTPase activity of rhabdomeric and washed rhabdomeric membranes. (a) [38 S]GTPgS binding to rhabdomeric membranes (B) or washed-rhabdomeric membranes (O) containing 1.6 pmoles Gqa was assessed for 0±60 s as described under Materials and methods. Reactions were stopped and [38 S]GTPgS bound to membranes was counted and plotted as a function of time. (b) the Gqa GTPase activity of rhabdomeric and washed-rhabdomeric membranes was determined by incubating membranes with [38 P]GTP for 0±60 s as described under Materials and methods. Reactions were quenched and the supermatants counted and plotted as a function of time. All values are the averages of duplicate reactions from representative experiments.

binding of 7.7/min and 0.23/min, respectively (data not shown). PLC-140 and other loosely associated proteins could be removed from the squid rhabdomeric membranes by washing in buffers containing 0.5 m NaCl (Fig. 2, W-Rhabs). We found no change in the GTPgS binding to the membranes following this washing procedure (Fig. 5a); however, there was a signi®cant decrease in the rate of GTP hydrolysis in the washed membranes compared with the original rhabdomeric membranes (Fig. 5b). Reconstitution of puri®ed endogenous PLC-140 with the washed membranes increased the GTPase activity ®vefold (Fig. 6a). When the stimulation of Gq GTPase activity was measured over a range of PLC-140 concentrations in the assay it was found that a molar ratio of PLC-140: Ga of 2: 1 was required in order to have any effect on GTPase activity.

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Fig. 6 Regulation of Gqa GTPase activity by PLC-140. (a) The Gqa GTPase activity in washed-rhabdomeric membranes containing 2.5 pmoles Gqa was measured in the absence (B) or presence (O) of 20 pmoles of puri®ed endogenous PLC-140 for 0±60 s as described under Materials and methods. 22 P was counted and pl percentage of the maximal GTP hydrolysis as a function of time. (b) The effect of PLC-140 on Gqa GTPase activity was measured in washed-rhabdomeric membranes. Increasing concentrations of puri-@ed PLC-140 were added to the membranes containing 2.5 pmoles Gqa and the GTPase reaction was initiated at time 0 by the addition of [n P]GTP. Reactions were stopped after 10 s by the addition of P counted and plotted as the percentage o perchloric acid and maximal GTP hydrolysis. Data points are the means of duplicate samples and are representative of two separate experiments.

This GAP activity increased to a maximal effect seen with a 10 ± 20 -fold molar excess of PLC (Fig. 6b).

Discussion

A cDNA that encodes the PLC enzyme we have previously isolated from squid rhabdomeric membranes was isolated in this study. This PLC protein was only found in the squid eye and not identi@ed in any other tissue from squid under the conditions that we have used in our study. PLC isozymes expressed in visual systems of other invertebrates have not been shown to have such speci@c tissue distribution. Lobster PLC-b was found to be widely expressed in many tissues (Xu and McClintock 1999) and Drosophila norpA, while predominantly expressed in the eye is also found in the optic

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Fig. 7 The role of PLC-140 regulation in visual transduction in squid photoreceptors. The model presents the molecular components of the squid visual system. Rhodopsin activation by light regulates GDP-GTP exchange on Gqa facilitating the Gq activation of PLC-140, PLC activation increases the production of inositol 1.4.5trisphosphate (IP3) and diacylglycerol (DAG) from inositol bisphosphate. IP3 stimulates the release of calcium from subrhabdomeric stores and the combination of calcium and DAG may lead to the opening of ion channels and depolarization of the membrane. Two reactions that limit the activation of this system are shown. First, the PLC-140 interaction with Goa-GTP increases the rate of GTP hydrolysis by the Gqa protein, limiting the time of PLC activation by the G protein. Second, the increase in calcium concentration stimulates a phosphodiesterase (PDE) in the squid photoreceptor leading to an increase in nucleotide concentrations that are inhibitory to PLC140 activity

lobe and brain (Bloomquist et al. 1988). Further identi@cation of the mRNA encoding the PLC enzyme and its expression in the various squid tissues is required to determine if the expression of the gene encoding PLC-140 is in fact eye-speci@c; however, the eye is clearly the site of highest expression of this protein.

The domain structure of PLC-140 was very similar to other PLC enzymes of the b family. As expected, the similarity between PLC-140 and other PLC-b enzymes is

and PLC-b2, and since we have shown that puri®ed PLC-140 is activated by calcium and squid Gq, they ma be sites for this regulation.

PLC-140 has a long carboxyl terminal region that contains the P-box and G-box motifs required for activa by G proteins (Lee et al. 1993; Park et al. 1993b; Wu et 1993a; Kim et al. 1996). The truncated squid PLC that w identi®ed by Carne et al. (1995) lacked a carboxyl-term tail and does not encode P- or G- box motifs and may therefore not be sensitive to Gga-dependent activation. support of this notion, we have seen cleavage of puri®e PLC-140 by exogenous calpain with loss of Gqa regula (L. H. Mayeenuddin, unpublished data). Although we h not been able to obtain amino acid sequence from calpa digested PLC-140 fragments, cleavage of the protein in . near the putative PEST sequence which spans amino ac 858±881, positive PEST score of 3.26 (Sekhar and Free 1998), would produce protein fragments of approximate the size observed by us and others (Suzuki et al. 1999). Carboxyl-terminal truncations have also been reported f mammalian PLC-b1, PLC-b3 and PLC-b4 (Park et al. 1993b; Banno et al. 1995; Kim et al. 1998). The effect o these truncations on PLC activation is enzyme-speci®c Cleavage of PLC-b3 by calpain renders the truncated PL more sensitive to regulation by G protein bg subunits (Banno et al. 1995), while the C-terminally truncated sp variant of PLC-b4, PLC-b4b, has been shown to be insensitive to regulation by both Gqa and Gbg (Kim et a 1998). The bg sensitivity of the calpain-digested PLC-1 has not been tested; however, given the lack of bg regulation of intact PLC-140 shown here, it seems unlik that the truncated protein will be bg-sensitive.

Two ATP/GTP binding sites were identi®ed within the deduced amino acid sequence of PLC-140. The GHOLTGKS sequence found within the X domain of PLC-140 (amino acids 344±351) and the AAKPVKGK sequence (amino acids 814±822) located between the C domain and the P- and G-boxes, both correspond to the (G/A)X4GK(S/T) identi®ed as one of the motifs found members of the GTPase superfamily (Bourne et al. 199 These are similar to the motifs found within PLC-b4 an norpA also within the X domains of these PLC isozyme (Lee et al. 1993). We have shown here that PLC-140 w able to bind guanine and adenine monophosphorylated,

greatest within the conserved X and Y catalytic domains (65±70% identity). These two regions are highly conserved amongst all PLC isozymes and they are essential for catalytic activity (Bristol et al. 1988; Ellis et al. 1993). The PH and C2 domains of PLC-140 represent structurally conserved protein modules, which in other PLC-b enzymes have been shown to be involved in diverse calcium and lipid interactions (Rizo and Sudhof 1998; Katan and Allen 1999). The PH and C2 domains of PLC-140 show 60±85% sequence similarity to the respective domains in PLC-b1

diphosphorylated and triphosphorylated nucleotides but cyclic nucleotides. Similar to PLC-b4 (Lee et al. 1994), guanine and adenine nucleotides inhibited the activity o PLC-140. It is possible that binding of nucleotides with the X domain could disrupt the catalytic activity of thes enzymes.

The lack of PLC-140 regulation by G protein bg subunits and inhibition by ribonucleotides, distinguishe this enzymes as a member of an emerging subgroup of PLC-b proteins involved in visual systems. It has been

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speculated that inhibition of PLC-b4 by monophosphate, diphosphate and triphosphate but not the cyclic nucleotides may be a mechanism for their regulation in the mammalian photoreceptors where the concentrations of GMP, GTP and cGMP are of course regulated by light (Lee et al. 1994). In the invertebrate visual systems, the major light-stimulated transduction pathway does not involve regulation of nucleotide hydrolysis, nevertheless, there have been reports of effects of guanine nucleotides in Limulus (Johnson et al. 1986) and squid (Saibil 1984) as well as a report of calciumstimulated cGMP phosphodiesterase activity in squid eyes (Brown and Kelman 1996). Possibly, in the invertebrate eye, light regulates nucleotide concentrations via a PLCstimulated rise in intracellular calcium and subsequent activation of cyclic nucleotide hydrolysis. Such a scheme is outlined in Fig. 7, where the nucleotide suppression of PLC activity is proposed as a mechanism to limit the activity of the PLC in the face of rising calcium levels and reduce the gain of the invertebrate visual system that occurs downstream of the PLC. If this mechanism exists then one would anticipate that inhibition of phosphodiesterase activity would lead to an increase in both the amplitude and duration of responses to light. Indeed, these were the ®ndings when the PDE inhibitor zaprinast was introduced into Limulus photoreceptors (Johnson and O'Day 1995). These authors found that the effect of zaprinast was only seen in response to stimulation by bright light, required calcium and was upstream of IP3 production. Clearly, there

are alternative explanations for these observations and more

phospholipids and Gq protein a subunits, consistent with our observations of the regulation of puri®ed PLC-140 protein. The demonstration that this protein can be inhibited by nucleotides and acts to limit the activity of its own stimulator, Gqa, suggests that PLC-140 plays a pivotal role in both the activation and inactivation of cephalopod visual systems.

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mus

work is needed to test if regulation of PLC by nucleotides does exist in vivo in an invertebrate visual system.

We have also examined the effect of PLC-140 on Gqa activity by regulation of its GTPase activity. We ®rst observed a change in GTPase activity of rhabdomeric membranes following removal of loosely associated proteins that include PLC-140. Reconstitution of puri®ed PLC-140 back to these membranes in molar excess of Gqa increased the GTPase activity ®vefold. The requirement for an excess of PLC to Gqa in order to signi@cantly in uence the rate of inactivation of Gqa is in keeping with our estimates of a threefold molar excess of total PLC-140 to total Gqa in the whole squid eye. It is not dif@cult to envision that an even greater concentration of PLC could occur at speci®c sites within the microvillar structure in response to light stimulation. Recent studies in Drosophila have also shown dependence of GTPase activity of dGq on the high concentrations of norp A PLC (Cook et al. 2000). It seems that feedback inhibition of invertebrate Gqa proteins by PLC is common to invertebrate visual systems and is required for single-photon responses in these systems.

In conclusion, we have cloned a cDNA encoding a PLC that appears to be the major effector of squid visual systems. This protein contains a number of identi@able domains that may be sites of interaction of PLC-140 with membranes,

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results of Dl

BLASTN 2.2.6 [Apr-09-2003]

RID: 1069187428-28318-68322664721.BLASTQ3

Query=

(7211 letters)

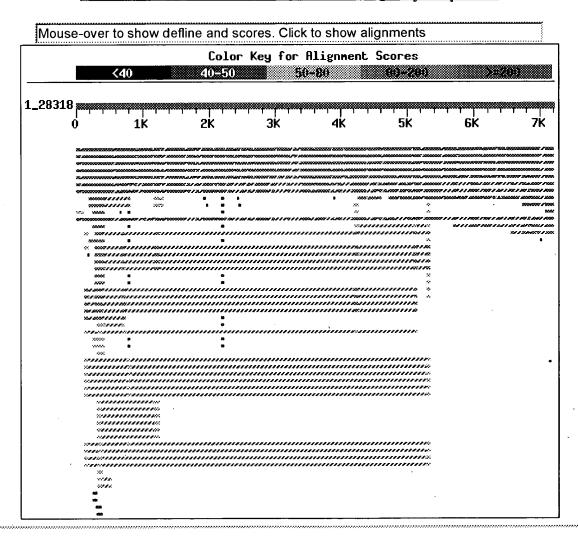
Database: All GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS,

GSS, or phase 0, 1 or 2 HTGS sequences)

1,968,555 sequences; 9,458,193,315 total letters

Taxonomy reports

Distribution of 1012 Blast Hits on the Query Sequence



Score Е

Sequences producing significant alignments:	(bits)	Value	•
gi 9507134 ref NM 019167.1 Rattus norvegicus beta-spectrin	383	e-102	
qi 11066460 gb AF225960.1 AF225960 Rattus norvegicus spectr	<u>383</u>	e-102	
gi 3550974 dbj AB008551.1 Rattus norvegicus mRNA for beta	383	e-102	
gi 3452552 dbj AB001347.1 Rattus norvegicus Spnb-3 mRNA fo	383	e-102	
gi 38084793 ref XM 129130.5 Mus musculus spectrin beta 3 (343	3e-90	
<pre>gi 5902121 ref NM 006946.1 Homo sapiens spectrin, beta, no</pre>	224	2e-54	
<pre>gi 29179634 gb BC048851.1 Mus musculus, Similar to beta-sp</pre>	224	2e-54	
<u>gi 23271110 gb BC033305.1 </u> Mus musculus, clone IMAGE:401041	224	2e-54	
gi 2511783 qb AF026489.1 AF026489 Mus musculus beta III spe	224	2e-54	
gi 3550976 dbj AB008567.1 Homo sapiens mRNA for beta-spect	224	2e-54 1e-49	
<u>gi 19879811 dbj AP001157.4 </u> Homo sapiens genomic DNA, chrom gi 2511780 qb AF026488.1 AF026488 Homo sapiens beta III spe	208		
<u>gi 2511780 gb AF026488.1 AF026488</u> Homo sapiens beta III spe gi 2511778 gb AF026487.1 AF026487 Homo sapiens beta III spe	<u>149</u> 149	8e-32	
gi 26090589 dbj AK044883.1 Mus musculus 9.5 days embryo pa	133	5e-27	
qi 22507315 ref NM 000347.3 Homo sapiens spectrin, beta, e	103	4e-18	
gi 24475587 ref NM 013675.2 Mus musculus spectrin beta 1 (103	4e-18	
qi 440899 qb S66283.1 S66283 Spnb-1=beta-spectrin [mice, re	1.03	4e-18	
gi 338439 gb J05500.1 HUMSPTB Human beta-spectrin (SPTB) mR	103	4e-18	
qi 34879631 ref XM 240072.2 Rattus norvegicus spectrin bet	101	2e-17	
qi 34866099 ref XM 234322.2 Rattus norvegicus similar to S	100	7e-17	
gi 7106420 ref NM 009260.1 Mus musculus spectrin beta 2 (S	94	4e-15	
gi 30348965 ref NM 175836.1 Mus musculus spectrin beta 2 (94	4e-15	
gi 26348244 dbj AK079842.1 Mus musculus 0 day neonate thym	94	4e-15	
qi 598330 gb M74773.1 MUSSPNA Mus musculus brain beta spect	94	4e-15	
gi 4102882 gb AF017112.1 AF017112 Mus musculus non-erythroc	94	4e-15	
gi 4102750 gb AF016040.1 AF016040 Mus musculus beta spectri	94	4e-15	
<pre>gi 4507194 ref NM 003128.1 Homo sapiens spectrin, beta, no</pre>	86	1e-12	
<u>qi 30315657 ref NM_178313.1 </u> Homo sapiens spectrin, beta, n	86	1e-12	
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<u>gi 27462179 gb AF327441.1 </u> Homo sapiens beta-spectrin 2 iso	86	1e-12	
gi 338442 qb:M96803.1 HUMSPTBN1A Human general beta-spectri	86		
<u>gi 425552 gb S65762.1 S65762</u> SPTBN1=beta-fodrin [human, mRN gi 22204389 emb AL672225.15 Mouse DNA sequence from clone	<u>76</u> 74	1e-09 4e-09	
gi 1657656 gb U73171.1 MMU73171 Mus musculus brain beta-spe	70	6e-08	
gi 18250160 gb AC093110.4 Homo sapiens BAC clone RP11-564H	66	1e-06	
<u>qi 13990322 emb AL121774.5 CNS01DSF</u> Human chromosome 14 DNA <u>qi 14625740 emb AL135745.5 CNS01DVD</u> Human chromosome 14 DNA	<u>66</u> 66	1e-06 1e-06	
gi 392912 qb U00910.1 HSU00910 Human beta-spectrin (HSPTB1)	66	1e-06	
gi 13435160 ref NM_025213.1 Homo sapiens spectrin, beta, n	64	4e-06	
gi 24642831 ref NM 078664.2 Drosophila melanogaster beta S	64	4e-06	
<u>gi 21734932 gb AC020929.6 </u> Homo sapiens chromosome 19 clone	64	4e-06	38'938
gi 22832735 gb AE003506.2 Drosophila melanogaster chromoso	64	4e-06	
<u>qi 11992161 qb AF311855.1 AF311855</u> Homo sapiens spectrin be <u>qi 14028959 qb AC012161.8 AC012161</u> Drosophila melanogaster,	<u>64</u> 64	4e-06 4e-06	
gi 14028958 gb AC011703.8 AC011703 Drosophila melanogaster,	64	4e-06	
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·			2000000000
<u>gi 3901275 gb AF079569.1 AF079569</u> Homo sapiens beta III spe	64	4e-06	
<pre>gi 11602889 gb AY004227.1 Homo sapiens betaIV spectrin iso</pre>	64		
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<u>gi 31201698 ref XM 309797.1 </u> Anopheles gambiae ENSANGP00000 gi 31201696 ref XM 309796.1 Anopheles gambiae ENSANGP00000	<u>62</u> 62	2e-05 2e-05	
gi 34855388 ref XM 218364.2 Rattus norvegicus similar to b		2e-03	
qi 30794219 ref NM 032610.1 Mus musculus spectrin beta 4 (<u>58</u> 58	2e-04	500000
		2e-04 2e-04	X00000X
gi 20372321 gb AY032694.1 Y032691S04 Mus musculus beta-spec	<u>58</u>	2e-04 2e-04	ROOOK
gi 16117404 gb AY032655.1 Mus musculus beta4-spectrin mRNA	58		
gi 18147601 dbj AB055618.1 Mus musculus mRNA for betaIV-sp	<u> 58</u>	0.001	2000002
gi 30160427 gb BC051562.1 Mus musculus cDNA clone IMAGE:15	<u> 56</u>	0.001	000000
<u>qi 20372320 gb AY032693.1 Y032691S03</u> Mus musculus beta-spec <u>qi 22204468 emb AL731792.12 </u> Mouse DNA sequence from clone	_ <u>56</u> _54	0.001	26228
qi 18859422 ref NM 131525.1 Danio rerio spectrin, beta, er	50	0.058	
gi 20372340 gb AY032713.1 Y032691S23 Mus musculus beta-spec	50	0.058	
gi 11992163 gb AF311856.1 AF311856 Homo sapiens spectrin be	50	0.058	
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gi 6453546 emb AL133093.1 HSM801370 Homo sapiens mRNA; cDNA	<u>50</u>	0.058	
gi 18147607 dbj AB055621.1 Mus musculus mRNA for betaIV-sp	<u>50</u>	0.058	
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gi 13540713 ref NM 022401.1 Rattus norvegicus plectin (Ple	48	0.23	
<u>gi 9581948 gb AC004965.2 </u> Homo sapiens PAC clone RP5-1106H1 gi 1292885 emb X59601.1 RNPLECT Rat mRNA for plectin	<u>48</u> 48	0.23	
gi 33879611 gb BC030158.2 Homo sapiens bromodomain contain	46	0.90	
gi 4505876 ref NM 000445.1 Homo sapiens plectin 1, interme	46	0.90	
gi 19718730 ref NM 058243.1 Homo sapiens bromodomain conta	46	0.90	
gi 7657217 ref NM 014299.1 Homo sapiens bromodomain contai	46	0.90	
gi 21321883 gb AC006459.3 Homo sapiens BAC clone GS1-281N8	46	0.90	***************************************
gi 34304491 gb AY356552.1 Canis familiaris beta-spectrin m	46	0.90	
<u>gi 30984902 gb AC109322.16 </u> Homo sapiens chromosome 8, clon <u>gi 2345059 gb AF013172.1 AH005869S01</u> Homo sapiens beta-spec	46 46	0.90 0.90	•
gi 23274170 gb BC035266.1 Homo sapiens, Similar to bromodo	46	0.90	
gi 28839047 gb BC047888.1 Homo sapiens, Similar to bromodo	46	0.90	***************************************
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<pre>gi 24658603 gb BC038988.1 Homo sapiens, Similar to bromodo</pre>	46	0.90	
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gi 1477645 gb U53204.1 HSU53204 Human plectin (PLEC1) mRNA,	46	0.90	
gi 1477649 qb U63610.1 HSPLEC1S3 Human plectin (PLEC1) gene	46	0.90	
gi 3184497 gb AC004798.1 AC004798 Homo sapiens chromosome 1	46	0.90	
gi 14704448 gb BC000156.1 BC000156 Homo sapiens, Similar to	<u>46</u> 46	0.90 0.90	
gi 1296661 emb Z54367.1 HSPLECTIN H.sapiens gene for plectin gi 16444668 emb AL139811.30 Human DNA sequence from clone	46	0.90	99468
gi 3115203 emb Y12059.1 HSHUNKI H.sapiens HUNKI mRNA	46	0.90	



qi 392908 qb U00906.1 HSU00906 Human beta-spectrin (HSPTB1)	46	0.90
qi 34495165 gb AC127350.3 Mus musculus chromosome 18 clone	44	3.6
gi 29293996 qb AC080129.26 Homo sapiens 3 BAC RP11-135A1 (44	3.6
gi 19747119 qb AC008533.9 Homo sapiens chromosome 5 clone	44	3.6
qi 26284723 gb AF550402.1 AF550396S7 Homo sapiens cob(I)ala	44	3.6
gi 15145575 gb AC053503.7 Homo sapiens BAC clone RP11-3160	44	3.6

Alignments

Get selected sequences Select all Deselect all Rattus norvegicus beta-spectrin 3 (Spnb3), mR Sqi[9507134|ref|NM 019167.1| Length = 8178Score = 383 bits (193), Expect = e-102Identities = 193/193 (100%) Strand = Plus / Plus Query: 193 aagtgggtgaactcccacctggcccgggtgacatgccgggtgggagacctgtacagcgac 252 Sbjct: 584 aagtgggtgaactcccacctggcccgggtgacatgccgggtgggagacctgtacagcgac 643 Query: 253 ctgcgggacgggcgcaacctcctgaggctcctggaggtgctctcgggagagaccctgcca 312 Sbjct: 644 ctgcgggacgggcgcaacctcctgaggctcctggaggtgctctcgggagagaccctgcca 703 Query: 313 aaacccaccaagggccggatgcggattcactgcctggagaatgtcgacaaagcactgcag 372 Sbjct: 704 aaacccaccaagggccggatgcggattcactgcctggagaatgtcgacaaagcactgcag 763 Query: 373 ttcctgaaggagc 385 1111111111111 Sbjct: 764 ttcctgaaggagc 776 Score = 367 bits (185), Expect = 2e-97Identities = 191/193 (98%) Strand = Plus / Plus Query: 1440 ggtgcaagcggtggacgccgtagccgcagaactggccgctgagcattaccatgacattaa 1499 Sbjct: 1831 ggtgcaagcggtggacgccgtagccgcagaactggccgctgagcactaccatgacattaa 1890 Query: 1500 gcgcattgcggcgcggcagaacaacgtggcccggctctgggacttcttacgagagatggt 1559 Sbjct: 1891 gcgcattgcggcgcggcagaacaacgtggcccggctctgggacttcttacgagagatggt 1950 Query: 1560 ggccgccgccgtgagcggctccttctcaacctggagctgcagaaggtgtttcaggacct 1619

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Strand = Plus / Plus
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Strand = Plus / Plus
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Query: 7091 gaaagagagcgagaaaaacgattcagcttctttaagaagaacaagtagttggggcaagac 7150
          Sbjct: 7511 gaaagagagcgagaaaaacgattcagcttctttaagaagaacaagtagttggggcaagac 7570
Query: 7151 teccaggecagetecetecetetgtteaggaaactgecagggaetgtegaeagagaeeae 7210
          Sbjct: 7571 tcccaggccagctccctccctctgttcaggaaactgccagggactgtcgacagagaccac 7630
Query: 7211 c 7211
Sbjct: 7631 c 7631
 Score = 222 \text{ bits (112)}, Expect = 7e-54
 Identities = 135/144 (93%), Gaps = 9/144 (6%)
 Strand = Plus / Plus
Query: 673 tttgagtccctgaagaagtgtaacgcacactacaatctgcagaatgctttcaatctggct 732
          Sbjct: 1064 tttgagtccctgaagaagtgtaacgcacactacaatctgcagaatgctttcaatctggct 1123
Query: 733 gaaaaggaacttggcctgacgaagctcctggatcct----aacgtagaccaaccc 783
Sbjct: 1124 gaaaaggaacttggcctgacgaagctcctggatcctgaagatgtgaacgtagaccaaccc 1183
Query: 784 gatgagaagtccatcatcacctac 807
          Sbjct: 1184 gatgagaagtccatcatcacctac 1207
Score = 204 bits (103), Expect = 2e-48
Identities = 126/135 (93%), Gaps = 9/135 (6%)
Strand = Plus / Plus
Query: 6551 atgccccagagcagatcgtctgagtcagctcatgttgccaccctgcccgcacgaggtgct 6610
          Sbjct: 6971 atgccccagagcagatcgtctgagtcagctcatgttgccaccctgcccgcacgaggtgct 7030
Query: 6611 gagctctctgctcaggaacagatggaaggg-----cgcaaacaggagatggaagcc 6661
Sbjct: 7031 gagctctctgctcaggaacagatggaagggacgctgtgccgcaaacaggagatggaagcc 7090
Query: 6662 ttcaataagaaagct 6676
          11111111111111
Sbjct: 7091 ttcaataagaaagct 7105
Score = 180 \text{ bits (91)}, Expect = 2e-41
Identities = 91/91 (100%)
```



results of Minas

BLASTX 2.2.6 [Apr-09-2003]

RID: 1069188234-32305-140862554207.BLASTQ3

Query=

(7211 letters)

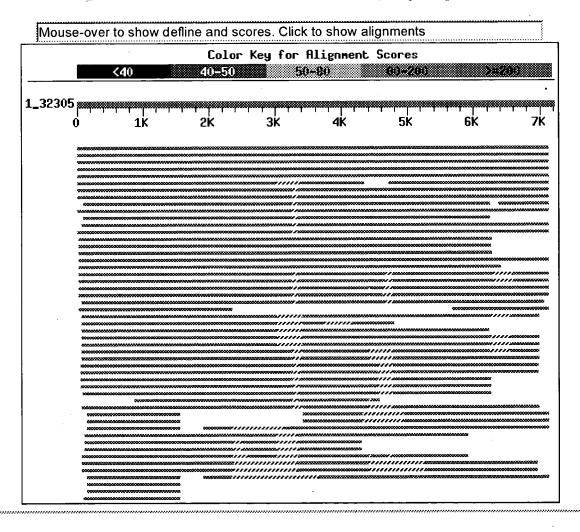
Database: All non-redundant GenBank CDS

translations+PDB+SwissProt+PIR+PRF

1,541,362 sequences; 503,870,249 total letters

Taxonomy reports

Distribution of 389 Blast Hits on the Query Sequence



Sequences producing significant alignments:

Score (bits) Value

		inne	
<pre>qi 11066461 qb AAG28596.1 spectrin-like protein GTRAP41 [R</pre>	1071	0.0	
<pre>gi 17367415 sp Q9QWN8 SPCP_RAT</pre> Spectrin beta chain, brain 2	1059	0.0	
<u>gi 38084794 ref XP 129130.3 </u> spectrin beta 3 [Mus musculus]	<u>1058</u>	0.0	
<pre>gi 9507135 ref NP_062040.1 beta-spectrin 3 [Rattus norvegi</pre>	1057	0.0	
<pre>gi 5902122 ref NP_008877.1 spectrin, beta, non-erythrocyti</pre>	<u>1018</u>	0.0	
gi 30348966 ref NP_787030.1 spectrin beta 2 isoform 1; bet	<u>711</u>	0.0	
<pre>gi 4507195 ref NP_003119.1 spectrin, beta, non-erythrocyti</pre>	709	0.0	
<pre>gi 30315658 ref NP 842565.1 spectrin, beta, non-erythrocyt</pre>	<u>699</u>	0.0	3
<pre>gi 448251 prf 1916380A</pre> beta spectrin (beta fodrin)	<u>698</u>	0.0	***
<u>gi 7106421 ref NP_033286.1 </u> spectrin beta 2 isoform 2; beta	696	0.0	
gi 34879632 ref XP 240072.2 similar to Spectrin beta chain	<u>696</u>	0.0	
<pre>gi 18859423 ref NP_571600.1 spectrin, beta, erythrocytic;</pre>	<u>577</u>	0.0	
gi 34866100 ref XP_234322.2 similar to Spectrin beta chain	<u>553</u>	0.0	
<pre>qi 27413156 ref NP 000338.2 spectrin, beta, erythrocytic (</pre>	<u>561</u>	0.0	
gi 134798 sp P11277 SPCB_HUMAN Spectrin beta chain, erythro	<u>560</u>	0.0	
gi 338440 gb AAA60578.1 spectrin Rouen (beta-220-218) muta	<u>561</u>	0.0	
<u>gi 7363453 ref NP 038703.1 </u> spectrin beta 1; beta-spectrin	<u>548</u>	0.0	
<u>qi 17647191 ref NP_523388.1 </u> beta Spectrin CG5870-PA [Droso	<u>520</u>	0.0	
qi 423777 pir A46147 spectrin beta chain - fruit fly (Dros	<u>520</u>	0.0	3
<u>qi 29179635 qb AAH48851.1 </u> Similar to beta-spectrin 3 [Mus <u>qi 28277312 qb AAH46267.1 </u> Similar to spectrin, beta, non-e	$\frac{721}{741}$	0.0	
gi 476928 pir A47213 beta-fodrin - human (fragment) >gi 42	423	0.0	**
qi 15213122 qb AAK85734.1 beta-G spectrin [Brugia malayi]	470	e-177	
gi 17976528 gb AAK77612.2 Uncoordinated protein 70, isofor	444	e-161	0000
<pre>gi 5734146 gb AAD49858.1 beta-G spectrin [Caenorhabditis e</pre>	444	e-161	000
<pre>gi 17562506 ref NP_504748.1 beta-G spectrin, UNCoordinated</pre>	444	e-161	
gi 25154697 ref NP 504749.2 beta-G spectrin, UNCoordinated	444	e-161	3
gi 7505762 pir T29140 hypothetical protein K11C4.3 - Caeno gi 31201697 ref XP 309796.1 ENSANGP00000012507 [Anopheles	<u>442</u> 517	e-161 e-144	
gi 31201699 ref XP 309797.1 ENSANGP00000023406 [Anopheles	517	e-144	
gi 226515 prf 1516310A beta spectrin	<u>461</u>	e-128	222
<pre>gi 13435161 ref NP_079489.1 spectrin, beta, non-erythrocyt</pre>	<u>461</u>	e-127 🔣	
<pre>gi 11602887 gb AAF93171.1 betaIV spectrin isoform sigma2 [</pre>	<u>461</u>	e-127	
<pre>qi 11602890 qb AAF93173.1 betaIV spectrin isoform sigma4 [</pre>	<u>459</u>	e-127	
gi 11992162 gb AAG42473.1 spectrin beta IV [Homo sapiens]	<u>458</u>	e−127 👢	3
gi 17368942 sp Q9H254 SPCQ HUMAN Spectrin beta chain, brain	<u>458</u>	e-127	***
gi 30794220 ref NP 115999.1 spectrin beta 4; beta-spectrin	<u>458</u>	e-127 e-127 	
<pre>gi 16117405 gb AAK38731.1 beta4-spectrin [Mus musculus] >g gi 338330 gb AAA63259.1 muscle beta spectrin</pre>	<u>458</u> 259	e-12/ % e-123	238
gi 34855389 ref XP 218364.2 similar to betaIV-spectrin sig	411	e-112	*
gi 2511779 gb AAC79502.1 beta III spectrin [Homo sapiens]	390	e-106	
gi 444793 prf 1908227A beta spectrin	$\frac{320}{320}$	2e-85	•••
qi 420115 pir S29854 spectrin beta chain - dog (fragment)	316	5e-84	
gi 18481635 gb AAL73492.1 beta I spectrin form betaI sigma	245	5e-75	***
gi 24656802 ref NP 523900.1 karst CG12008-PA [Drosophila m	<u>258</u>	9e-67	***
<pre>gi 7655 emb CAA37939.1 betaH spectrin [Drosophila melanoga gi 103502 pir A37792 spectrin beta-H chain - fruit fly (Dr</pre>	<u>258</u> 258	9e-67 ‱ 1e-66	23
<u>qi 31209523 ref XP 313728.1 </u> ENSANGP00000017034 [Anopheles	<u>250</u>	2e-64	

<u>gi 25155104 ref NP 741632.1 </u> SMAll body size SMA-1, betaH c <u>gi 7506776 pir T23630</u> hypothetical protein R31.1 - Caenorh	<u>246</u> 246	6e-63
gi 13123941 sp Q9QXQ0 AAC4 RAT Alpha-actinin 4 (Non-muscle	<u>213</u>	4e-53
gi 11230802 ref NP 068695.1 actinin alpha 4 [Mus musculus]	<u>213</u>	5e-53
gi 38197444 gb AAH61786.1 Actn4 protein [Rattus norvegicus]	213	5e-53
gi 12025678 ref NP 004915.2 actinin, alpha 4 [Homo sapiens	212	7e-53
gi 2804273 dbj BAA24447.1 alpha actinin 4 [Homo sapiens] gi 2493432 sp Q90734 AAC4 CHICK Alpha-actinin 4 (Non-muscle gi 32766291 gb AAH54901.1 Unknown (protein for MGC:63508)	212 211 207	7e-53 2e-52 2e-51
gi 24639238 ref NP 477484.2 alpha actinin CG4376-PA [Droso	204	2e-50
gi 13928936 ref NP 113863.1 alpha actinin 4; alpha actinin	204	2e-50
gi 17565034 ref NP 506128.1 actinin (104.1 kD) (atn-1) [Ca	<u>203</u>	3e-50
gi 24639240 ref NP 726784.1 alpha actinin CG4376-PB [Droso	<u> 202</u>	7e-50
<pre>gi 13124689 sp P18091 AACT_DROME Alpha-actinin, sarcomeric gi 1070611 pir FAFFAA alpha-actinin - fruit fly (Drosophil gi 31242387 ref XP 321624.1 ENSANGP00000011796 [Anopheles gi 2511781 gb AAC79503.1 beta III spectrin [Homo sapiens]</pre>	202 201 201 201	1e-49 1e-49 2e-49 2e-49
gi 8186 emb CAA36042.1 unnamed protein product [Drosophila	200	3e-49
gi 3157976 gb AAC17470.1 alpha actinin [Homo sapiens]	199	5e-49
<u>gi 17137758 ref NP 477485.1 </u> alpha actinin CG4376-PC [Droso <u>gi 25992501 gb AAN77132.1 </u> alpha-actinin [Danio rerio] >gi	<u>198</u> 198	1e-48
gi 211077 gb AAA48567.1 actinin [Gallus gallus] gi 13124665 sp P05094 AAC1 CHICK Alpha-actinin 1 (Alpha-act	<u>196</u> 196	7e-48
gi 539494 pir A42162 alpha-actinin 1 - chicken >gi 211083	196	7e-48
<u>gi 32766313 qb AAH54911.1 </u> Unknown (protein for MGC:63559) <u>gi 37362178 qb AAQ91217.1 </u> actinin, alpha 2 [Danio rerio]	<u>196</u> <u>195</u>	7e-48 9e-48
<pre>gi 38018016 qb AAR08137.1 brain-specific alpha actinin 1 i</pre>	<u>193</u>	3e-47
<u>gi 13591902 ref NP 112267.1 </u> actinin, alpha 1; non-muscle a <u>gi 30585329 gb AAP36937.1 </u> Homo sapiens actinin, alpha 1 [s	<u>193</u> <u>192</u>	3e-47 1e-46
gi 4501891 ref NP 001093.1 actinin, alpha 1 [Homo sapiens] gi 32766260 gb AAH54830.1 Unknown (protein for MGC:62771)	<u>192</u> 192	1e-46
gi 112959 sp P12814 AAC1 HUMAN Alpha-actinin 1 (Alpha-actin	<u>192</u>	1e-46
gi 63389 emb CAA32079.1 fibroblast alpha actinin [Gallus g gi 23394914 gb AAN31639.1 alpha-actinin [Biomphalaria glab	192 191	1e-46 2e-46
<u>gi 28193204 emb CAD62344.1 </u> unnamed protein product [Homo s gi 34876265 ref XP 214499.2 similar to actinin, alpha 2 [R	<u>191</u> <u>189</u>	2e-46 5e-46
gi 34876265 ref XP 214499.2 similar to actinin, alpha 2 [R gi 4501893 ref NP 001094.1 actinin, alpha 2 [Homo sapiens]	189	5e-46
gi 31981445 ref NP 150371.2 actinin alpha 2 [Mus musculus] gi 32449722 qb AAH43995.2 Similar to actinin, alpha 1 [Xen	189 188	6e-46 11
gi 4557241 ref NP 001095.1 skeletal muscle specific actini gi 112955 sp P20111 AAC2 CHICK Alpha-actinin 2 (Alpha actin	187 187	2e-45 2e -45
gi 19173800 ref NP 596915.1 actinin alpha 3 [Rattus norveg	185	9e-45
gi 7304855 ref NP 038484.1 actinin alpha 3 [Mus musculus]	185	9e-45
gi 20853961 gb AAM26632.1 truncated alpha-actinin [Rattus	185	9e-45
gi 13123947 sp Q9JI91 AAC2 MOUSE Alpha-actinin 2 (Alpha act	184	2e-44
gi 555419 gb AAA48566.1 alpha-actinin	1.84	3e-44
<u>gi 7706190 ref NP 057726.1 </u> spectrin, beta, non-erythrocyti <u>gi 31242389 ref XP 321625.1 </u> ENSANGP00000023605 [Anopheles	182 182	8e-44 1e-43
<u>gi 2137272 pir 149290</u> dystonin - mouse (fragment) >gi 9040	174	3e-41



 gi|19882219|ref|NP 598594.1|
 dystonin isoform a; bullous pe...
 174
 3e-41

 gi|19882221|ref|NP 604443.1|
 dystonin isoform b; bullous pe...
 174
 3e-41

 gi|30315911|sp||Q60824 1
 [Segment 1 of 2] Bullous pemphigoi...
 173
 4e-41

Alignments

G	et selei	cted sequences Select all Deselect all	
[]> <u>gi </u>		spectrin-like protein GTRAP41 [Rattus ength = 2388	norvegicus]
	ities	71 bits (2770), Expect(3) = 0.0 = 750/1295 (57%), Positives = 760/1295 (58%), Gaps = 210/1295	5 (16%)
Query:	1	MSSTLSPTDFDSLEIQWDLPDSDLFERSRIRQYSDINNRSSS MSSTLSPTDFDSLEIQ WDLPDSD LFERSRI+ +D	126
Sbjct:	1	MSSTLSPTDFDSLEIQGQYSDINNRWDLPDSDWDNDSSSARLFERSRIKALAD	53
Query:		ARDEREAVQKKTFTWDNDKALAKWVNSHLARVTCRVGDLYSXXXXXXXXXXXXXEVLSGET EREAVQKKTFT KWVNSHLARVTCRVGDLYS EVLSGET	
Sbjct:	54	EREAVQKKTFTKWVNSHLARVTCRVGDLYSDLRDGRNLLRLLEVLSGET	102
Query:	307	LPKPTKGRMRIHCLENVDKALQFLKELENMGSHDIVDGQKVHNHRLTLGLVWTIIL LPKPTKGRMRIHCLENVDKALQFLKE LENMGSHDIVDG NHRLTLGLVWTIIL	474
Sbjct:	103	LPKPTKGRMRIHCLENVDKALQFLKEQKVHLENMGSHDIVDGNHRLTLGLVWTIIL	158
Query:	475	RFEDNKEKKCQMKTAGQIQDISVETDALLLWVNVHNFSAKY- RF EDNKEKK CQMKTAG VNVHNF+ +	597
Sbjct:	159	RFQIQDISVETEDNKEKKSAKDALLLWCQMKTAGYPNVNVHNFTTSWR	206
Query:	598	PNLAFNAIVHKHRPDLLDTTSWRDGFESLKKCNAHYNLQNAFNLAEKELGLTKLLDP LAFNAIVHKHRPDLLD FESLKKCNAHYNLQNAFNLAEKELGLTKLLDP	768
Sbjct:	207	DGLAFNAIVHKHRPDLLDFESLKKCNAHYNLQNAFNLAEKELGLTKLLDPEDV	259
Query:	769	NVDQPDEKSIITYKMKALAVEDVVSTYYHYFSRIGKVLDHAMEAEASELL NVDQPDEKSIITY V+TYYHYFS RIGKVLDHAMEAE	918
Sbjct:	260	NVDQPDEKSIITYVATYYHYFSKMKALAVEGKRIGKVLDHAMEAE	304
Query:	919	QWEGKHLVEKYESLTIGTFNDRQLANSVQNQLQSFNSYRTIEQLSGV HLVEKYESL TIGTFNDRQLANS VQNQLQSFNSYRT V	1059
Sbjct:	305	HLVEKYESLASELLQWIEQTIGTFNDRQLANSLSGVQNQLQSFNSYRTV	353
Query:	1060	EKPPKFTEKGNLEVLIQSKLRANNQKVYEGRLILFTTPRSDINKAWERLEKAEH EKPPKFTEKGNLEVL IQSKLRANNQKVY EGRLI SDINKAWERLEKAEH	1221
Sbjct:	354	EKPPKFTEKGNLEVLLFTIQSKLRANNQKVYTPREGRLISDINKAWERLEKAEH	407
Query:	1222	ERELALRTEAARFDRENQRLVLIRQEKLEQLRETWLSFGL ERELALRTE LIRQEKLEQL RETWLS FGL	1341
Sbjct:	408	ERELALRTELIRQEKLEQLAARFDRKAAMRETWLSENQRLVSQDNFGL	455
Query:	1342	ELAKAAMSQDNRKHEAIETDIVAYSGRXXXXXXXXXXXXXXXXXXXXXXHYHDIKRIAARQN EL AA+ RKHEAIETDIVAYSGR HYHDIKRIAARON	1521
Sbjct:	456	ELAAVEAAVRKHEAIETDIVAYSGRVQAVDAVAAELAAEHYHDIKRIAARQN	507

Query:	1522	NVARLWDFLREMVAAXXXXXXXXXXXXXQKVFQDLLYLMQSQDLGQLHELVDWMAEMKGRL- NVARLWDFLREMVAA QKVFQDLLYLM DWMAEMKGRL	1698
Sbjct:	508		555
Query:	1699	KHLAGVEDLLEYIAVQAERVRAVSAYRPCGEQSYESALRFCDPGKEXX KHLAGVEDLL+ IAVQAERVRAVSA SALRFCDPGKE	1842
Sbjct:	556	SQDLGKHLAGVEDLLQLHELVEADIAVQAERVRAVSASALRFCDPGKEYR	605
Query:	1843	XXXXXXXXXXPQLVSALCELRRLWRFLWEVGEAEAWVREQQHLLA Q ALCEL RRLWRFLWEVGEAEAWVREQQHLLA	1977
Sbjct:	606	PCGPQLVSERVATLEQSYEALCELAATRRARLEESRRLWRFLWEVGEAEAWVREQQHLLA	665
Query:	1978	SAETGRDLTGVLRLLNKHTALRGLTLEQGNQASTEMSGRLGPLKQQLVAEGHPG SAETGRDLTGVLRLLNKHTALRG EMSGRLGPLK QQLVAEGHPG	2139
Sbjct:	666	SAETGRDLTGVLRLLNKHTALRGEMSGRLGPLKLTLEQGQQLVAEGHPG	714
Query:	2140	ALQXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	2286
Sbjct:	715	~ ~ ~	768
Query:	2287	LVSSPEVGHDEFSTQARALEEEIXXXXXXXXXXXXQHPTLDALREQAA LVSSPEVGHDEFSTQA RALEEEI PTLDALREQAA	2430
Sbjct:	769	LVSSPEVGHDEFSTQALARQHRALEEEIRAHRPTLDALREQAAALPPALS	818
Query:	2431	EVQGRVPTLEQRARALLSHTPHYEELQARAGEFYTMLSEPGACNXXX EVQGRVPTLEQ HYEELQARAGE FYTMLSE GAC	2571
Sbjct:	819		865
Query:	2572	XXXXXXXWVEEKEQWLDPEVVQQRFETITAVSPERLELEPEMNALAAR-WVEEKEQWL DPEVVQQRFET LEPEMNALAAR	2718
Sbjct:	866	LWVEEKEQWLNGLALPERLEDPEVVQQRFETLEPEMNALAARI	908
Query:	2719	DIAEQLLKASPPGKDRIIGTQEQLNQRWQQFRSLAGGKKAALTSALSI DIAEQLLKASPPGKDRIIGTQEQLNQRWQQFRSLAGGKKAALTSALSI	2862
Sbjct:	909	TAVSDIAEQLLKASPPGKDRIIGTQEQLNQRWQQFRSLAGGKKAALTSALSIQNYHLECT	968
Query:	2863	ETQAWMREQDLGNDLAQNYHLECTKTKVIESTGVLALQRKLAGTERDL*ADP ETQAWMRE QDLGNDLA GVLALQRKLAGTERDL A	3018
Sbjct:	969	ETQAWMREKTKVIESTQDLGNDLAGVLALQRKLAGTERDLEA-I	1011
Query:	3019	RAK*SHLCPGGFGCWAPSPSPCHQHTMGGSSGNPGLARRLGEVQTMRRREES A+ L A P+ P + RLGEVQ TMRRREES	3174
Sbjct:	1012	SARVGELTQEANALAAGHPAQAPAINTRLGEVQTGWEDLRATMRRREES	1060
Query:	3175	DFLRSLDDFQAWLGRRLQTQTAVASYE 3255 DFLRSLDDFQAWLGR TQTAVAS E	
Sbjct:	1061	LGEARRLQDFLRSLDDFQAWLGRTQTAVASEE 1092	
	ities	40 bits (1910), Expect(3) = 0.0 = 536/931 (57%), Positives = 545/931 (58%), Gaps = 144/931 (3	L5%)
Query:	4775	QAWMGEQEKAKDELSAQVLEQALALHMMGQEQAEVKKHDYAQTIKQ +AWMGEQE KAKDELSAQ VLEQALA DYAQTIKQ	4912
Sbjct:	1601	EAWMGEQELHMMGQEKAKDELSAQAEVKKHQVLEQALADYAQTIKQ	1646
Query:	4913	LAASSQDMIDHEHPESTRLTIRGLKELAQAQVDKLYARERLQEHLRLCQXX LAASSQDMIDHEHPESTRLTIR QAQVDKLYA RERLQEHLRLCQ	5065

Sbjct:	1647	LAASSQDMIDHEHPESTRLTIRQAQVDKLYAGLKELAGERRERLQEHLRLCQLR	1700
Query:	5066	XXXXXXXXXXXXXSHELGQDYEHEQWIQERVTMLRDKFREFSRDTDSXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	5230
Sbjct:	1701	RELDDLEQWIQEREVVAASHELGQDYEHVTMLRDKFREFSRDT	1743
Query:	5231	XTSTIGQERVNGLIAGGHEWKDSLNEAWADLLEVALLDTRGQVLAA STIGQERV NGLIAGGH EWKDSLNEAWADLLE LLDTRGQVLAA	5368
Sbjct:	1744	STIGQERVDSANALANGLIAGGHAAWATVAEWKDSLNEAWADLLELLDTRGQVLAA	1799
Query:	5369	AYELQRFLHGARQALARVQHKAAEALQRQQQLPDGTGRDLNEHDD-DGRHCAYVQQVQIQ	5545
Sbjct:	1800	AYELQRFLHGARQALARVQHK QQQLPDGTGRDLN + RHCAY + IQ AYELQRFLHGARQALARVQHKQQQLPDGTGRDLNAAEALQRRHCAYEHDIQ	1850
Query:	5546	ALSTQLRLQKAYAGDKAQEIQLQTTDGRHMQXXXXXXXXXXXRRQLLL	5692
Sbjct:	1851	ALSTQ LRLQKAYAGDKA+EI GRHMQ RRQLLL ALSTQVQQVQDDGLRLQKAYAGDKAEEIGRHMQAVAEAWAQLQGSSAARRQLLL	1904
Query:	5693	DKFRFFKAVRELMLWMAQERPRDKNQQGIKDGINLQMDVSSADLVI DKFRFFKAVRELMLWM DGINLQM DVSSADLVI	5830
Sbjct:	1905	DKFRFFKAVRELMLWM DGINLQM DVSSADLVI DTTDKFRFFKAVRELMLWMDGINLQMDAQERPRDVSSADLVIKNQQ	1950
Query:	5831	AEIEARADRFSACIDRNHYAAEELQSRRMGQELLAISEKLSQ	5956
Sbjct:	1951	AEIEARADRFSACID RNHYAAEE ISEKLSQ GIKAEIEARADRFSACIDMGQELLARNHYAAEEISEKLSQLQSRRQET	1998
Query:	5957	AEKWQEKMDWXXXXXXXXXXXXFGRDAGMAEAWLGCEAFLCSQEPLVRSAE	6109
Sbjct:	1999	AEKWQEKMDW FGRDAGMAEAW LCSQEPLVRSA E AEKWQEKMDWLQLVLEVLVFGRDAGMAEAWLCSQEPLVRSAELGCTVDEVE	2049
Query:	6110	SLIKRHTVDEVEQKSAVAWEERFSALEKLTALQERENEQKRKPTSEPRYEE*RRKQP SLIKRH E QKSAVAWEERFSALEKLTAL+ERENEQKRK R EE RRKQP	6280
Sbjct:	2050	SLIKRHEAFQKSAVAWEERFSALEKLTALEERENEQKRKREEEERRKQPPTS	2101
Query:	6281	PEGSLVDGQRVXHPT-QSMASQLDTATQAPSINGVCTDKLPPSTESSQPLLEQQ PEGSLVDGQRV S+L +TQAPSINGVCTD TESSQPLLEQQ	6439
Sbjct:	2102	EPMASQPEGSLVDGQRVLDTAWDGTQSKLPPSTQAPSINGVCTDTESSQPLLEQQ	2156
Query:	6440	RLEQSNVEXXXXXXXXXXXXXXXXXDRVERQTLPRMPQSRSSESAHVATLPA RLEQSNV R ERQTLPR MPQSRSSESAHVATLPA	6601
Sbjct:	2157	RLEQSNVPEGPGSGTGDESSGPRGERQTLPRGPAPSPMPQSRSSESAHVATLPA	2210
Query:	6602	RGAELSAQEQMEGRKQEMEAFNKKAYCVLRRGTLCANRSWQNVSLGFYKD RGAELSAQEQMEG RKQEMEAFNKKA ANRSWQNV SLGFYKD	6751
Sbjct:	2211	RGAELSAQEQMEGTLCRKQEMEAFNKKAANRSWQNVYCVLRRGSLGFYKD	2260
		ARAASAGVSLARAQGKRKHVFKLPYHGEVPVSVAFDYRGLQDGKE	
Sbjct:	2261	ARAASAGV PYHGEVPV SVAFDYR GLQDGKE ARAASAGVPYHGEVPVSLARAQGSVAFDYRKRKHVFKLGLQDGKE	2305
Query:	6887	YLFQARVVNAAIAKDEAEMSSWLTASSASGXXXXXXXXXXDGRTRAMTMIV YLFQ AKDEAEMSSWL TASSASG G TRAMTM	7042
Sbjct:	2306	YLFQ AKDEAEMSSWL TASSASG G TRAMTM YLFQAKDEAEMSSWLRVVNAAIATASSASGEPEEPVVPSASRGLTRAMTMPP	2357
Query:	7043	LRSKSASRGLSQPEGSEREREKRFSFFKKNK 7135 + S L +G EREREKRFSFFKKNK	
Sbjct:	2358	VSQPEGSIVLRSKDGREREREKRFSFFKKNK 2388	

```
Score = 496 \text{ bits } (1278), \text{ Expect}(3) = 0.0
Identities = 347/585 (59%), Positives = 356/585 (60%), Gaps = 74/585 (12%)
Frame = +3
Query: 3258 PSTLPRGETL---GXXXXXXXXXXXXXXXXXSRLR-----QADPQCVERAQSEEVTRDR 3401
          P+TLP E L YSRLR QADPQC+ R
Sbjct: 1094 PATLPEAEALLAQHAALRGEVERAQSEYSRLRTLGEEVTRDQADPQCLFL-----R 1144
Query: 3402 QRLEALGTGWEELGLFLRMWESRQGRLAQAHGFQGFLRDARQAEGVLTEMPGTKLEDFMS 3581
          QRLEALGTGWEELG RMWESRQGRLAQAHGFQGFLRDARQAEGVL S
Sbjct: 1145 QRLEALGTGWEELG---RMWESRQGRLAQAHGFQGFLRDARQAEGVL------S 1189
Query: 3582 SQEYVLSH-----LQAADAAIK-----STMDANGERIRGLLEAGRQLVSKGNIHAEKI 3725
                      LQAADAAIK STMDANGERIRGLLEAGRQLVSKGNIHAEKI
          SQEYVLSH
Sbjct: 1190 SQEYVLSHTEMPGTLQAADAAIKKLEDFMSTMDANGERIRGLLEAGRQLVSKGNIHAEKI 1249
Query: 3726 QEKADSIEKRHRKNQEAVQQLLGRLRDNR----FLQDCQELKLWVSYDEEQQHIDEKMLT 3893
          QEKADSIEKRHRKNQEAVQQLLGRLRDNR FLQDCQELKLW IDEKMLT
Sbjct: 1250 QEKADSIEKRHRKNQEAVQQLLGRLRDNREQQHFLQDCQELKLW-----IDEKMLT 1300
Ouery: 3894 AQD------HTKWQKHQAFMDKVDKARNLAELAANKDWL-----LTLEKPELK 4019
        AQD HTKWQKHQAFM AELAANKDWL LTLEKPELK
Sbjct: 1301 AQDVSYDEARNLHTKWQKHQAFM------AELAANKDWLDKVDKEGRELTLEKPELK 1351
Query: 4020 VLRWDELEGREVSEKLEDLHR-----QAKARSLFDANALESWETTTRAELFAQSCS 4172
          VL VSEKLEDLHR QAKARSLFDAN RAELFAQSCS
Sbjct: 1352 VL------VSEKLEDLHRRWDELETTTQAKARSLFDAN-----RAELFAQSCS 1393
Query: 4173 ----LESLQAQLHSDDYGKDLTSVNILLKKQQMLEREMAVR*KEVEAIQAQAKALAQED 4337
               LESLQAQLHSDDYGKDLTSVNILLKKQQMLEREMAVR KEVEAIQAQAKALAQED
Sbjct: 1394 ALESWLESLQAQLHSDDYGKDLTSVNILLKKQQMLEREMAVREKEVEAIQAQAKALAQED 1453
Query: 4338 QSAGE----SRAVEEKFRALLQ--ASR*KDLSA-HEXXXXXXXXXXXXXXXTVGAAPGDRA 4496
                   SRAVEEKFRAL Q R + L A E
Sbjct: 1454 QSAGEVERTSRAVEEKFRALCQPMKDRCRRLQASREQHQFHRDVEDEILWVTERLPMASS 1513
Query: 4497 ASHGQLSGAWQEEKPDSAPDCRPQRLAQRPASHDPGP*AHSEN-----TEGDRQAELQ 4655
            HG+ + Q + Q L + H+P - E · G
Sbjct: 1514 LEHGKDLPSVQLLMK-----KNQTLQKEIQGHEPRIADLKERQRTLRTAAAGPELAELQ 1567
Query: 4656 EMWKRLSHELEAAAGPELLRGKRLEEALRAQQFYRDAAEARLGWG 4790
          EMWKRLSHELE LRGKRLEEALRAQQFYRDAAEA
Sbjct: 1568 EMWKRLSHELE-----LRGKRLEEALRAQQFYRDAAEAEAWMG 1605
Score = 80.5 bits (197), Expect = 4e-13
Identities = 54/98 (55%), Positives = 56/98 (57%), Gaps = 26/98 (26%)
Frame = +1
Query: 4411 CQPMKDRCRR-----FHRDVEDEILWEQHQVTERLPMASSLEHGK-----KK 4536
          CQPMKDRCRR FHRDVEDEILW VTERLPMASSLEHGK
Sbjct: 1474 CQPMKDRCRRLQASREQHQFHRDVEDEILW----VTERLPMASSLEHGKDLPSVQLLMKK 1529
Query: 4537 NQTL-----PRIADLKDLPSVQLLMIQGHERTLRT 4626
          NQTL PRIADLK+
Sbjct: 1530 NQTLQKEIQGHEPRIADLKE-----RQRTLRT 1556
```

```
Score = 37.4 \text{ bits } (85), \text{ Expect = } 4.1
Identities = 41/168 (24%), Positives = 72/168 (42%), Gaps = 13/168 (7%)
 Frame = +2
Ouery: 5675 RRQLLLDKFRFFKAVRELMLWMAQERPRDKNQQGIKDGINLQMDVSSADLVIAEIEARAD 5854
           RQ +++F+V++LW++P++KD++Q+++EI+
Sbjct: 1483 RLQASREQHQFHRDVEDEILWVTERLPMASSLEHGKDLPSVQLLMKKNQTLQKEIQGHEP 1542
Query: 5855 RFSACIDRNHYAAE-----ELQS--RRMGQELLAISEKLSQAEKWQEKMDWXXXXXX 6004
                               ELQ +R+ EL
                                                ++L +A + Q+
Sbjct: 1543 RIADLKERQRTLRTAAAGPELAELQEMWKRLSHELELRGKRLEEALRAQQ----- 1592
Query: 6005 XXXXXXFGRDAGMAEAWLG-CEAFLCSQEPL--VRSAESLIKRHTVDE 6139
                F RDA AEAW+G E + QE SA++ +K+H V E
Sbjct: 1593 -----FYRDAAEAEAWMGEQELHMMGQEKAKDELSAQAEVKKHQVLE 1634
Score = 37.0 \text{ bits } (84), \text{ Expect} = 5.4
Identities = 70/325 (21%), Positives = 125/325 (38%), Gaps = 15/325 (4%)
Query: 3405 RLEALGTGWEELGLFLRMWESRQGRLAQAHGFQGFLRDARQAEGVLTEMPGTKLEDFMSS 3584
           R L WE L E R RLAQA F DA E L +
Sbjct: 721 RAAELQAQWERLEALA---EERAQRLAQAASLYQFQADANDMEAWLVDALRLVSSPEVGH 777
Query: 3585 QEYVLSHLQAADAAIKSTMDANGERIRGLLEAGRQLVSKGNIHAEKIQEKADSIEKRHRK 3764
           E+ L A++ + A+ + L E L + H ++Q + ++E+ + +
Sbjct: 778 DEFSTQALARQHRALEEEIRAHRPTLDALREQAAALPPALS-HTPEVQGRVPTLEQHYEE 836
Query: 3765 NQ----EAVQQLLGRLRDNRFLQDCQELKLWVSYDEE-----QQHIDEKMLTAQDHT 3908
            Q \quad E \quad + \quad L \qquad L \quad L \quad + \qquad LWV \quad E + \quad
                                                     ++ D +++ + T
Sbjct: 837 LQARAGERARALEAALAFYTMLSEAGACGLWVEEKEQWLNGLALPERLEDPEVVQQRFET 896
Query: 3909 KWQKHQAFMDKVDKARNLAELAANKDWLLTLEKPELKVLRWDELEGREVSEKLEDLHRQA 4088
                         ++AE LL P + + + ++
             + A ++
Sbjct: 897 LEPEMNALAARITAVSDIAEQ-----LLKASPPGKDRIIGTQEQLNQRWQQFRSLAGGK 950
Query: 4089 KARSLFDANALESWE---TTTRAELFAQSCSLESLQAQLHSDDYGKDLTSVNILLKKQQM 4259
           KA +L A +++++ T T+A + ++ +ES Q D G DL V
Sbjct: 951 KA-ALTSALSIQNYHLECTETQAWMREKTKVIESTQ-----DLGNDLAGV-----LA 996
Query: 4260 LEREMAVR*KEVEAIQAQAKALAQE 4334
           L+R++A +++EAI A+ L QE
Sbjct: 997 LQRKLAGTERDLEAISARVGELTQE 1021
Score = 36.6 \text{ bits } (83), \text{ Expect = } 7.0
Identities = 39/141 (27%), Positives = 58/141 (41%), Gaps = 4/141 (2%)
Frame = +1
Query: 1636 SQDLGQLHELVDWMAEMKGRLKHLAGVEDLLEYIAVQA----ERVRAVSAYRPCGEQSYE 1803
           +QDLG + +A + + LAG E LE I+ + + A++A P
Sbjct: 984 TQDLG-----NDLAGVLALQRKLAGTERDLEAISARVGELTQEANALAAGHPAQAPAIN 1037
Query: 1804 SALRFCDPGKEXXXXXXXXXXXPQLVSALCELRRLWRFLWEVGEAEAWVREQQHLLASA 1983
           + L GE
                                     +L E RRL FL + + +AW+ Q +AS
Sbjct: 1038 TRLGEVQTGWEDLRATMRRRE-----ESLGEARRLQDFLRSLDDFQAWLGRTQTAVASE 1091
```

Query: 1984 ETGRDLTGVLRLLNKHTALRG 2046
E L LL +H ALRG
Sbjct: 1092 EGPATLPEAEALLAQHAALRG 1112

Spectrin beta chain, brain 2 (Spectrin, non-eryt (Beta-III spectrin) (SPNB-3) (Beta SpIII sigma 1) (Spectrin-like protein GTRAP41) gi|3550975|dbj|BAA32699.1| beta-spectrin III [Rattus norvegicus] Length = 2388Score = 1059 bits (2738), Expect(3) = 0.0Identities = 746/1295 (57%), Positives = 756/1295 (58%), Gaps = 210/1295 (16%) Query: 1 MSSTLSPTDFDSLEIQ-----WDLPDSD-----LFERSRIRQYSDINNRSSS 126 MSSTLSPTDFDSLEIQ WDLPDSD LFERSRI+ +D Sbjct: 1 MSSTLSPTDFDSLEIQGQYSDINNRWDLPDSDWDNDSSSARLFERSRIKALAD----- 53 Query: 127 ARDEREAVQKKTFTWDNDKALAKWVNSHLARVTCRVGDLYSXXXXXXXXXXXXXXEVLSGET 306 EREAVQKKTFT KWVNSHLARVTCRVGDLYS Sbjct: 54 ---EREAVQKKTFT------KWVNSHLARVTCRVGDLYSDLRDGRNLLRLLEVLSGET 102 Query: 307 LPKPTKGRMRIHCLENVDKALQFLKE----LENMGSHDIVDGQKVHNHRLTLGLVWTIIL 474 LPKPTKGRMRIHCLENVDKALQFLKE LENMGSHDIVDG NHRLTLGLVWTIIL Sbjct: 103 LPKPTKGRMRIHCLENVDKALQFLKEQKVHLENMGSHDIVDG----NHRLTLGLVWTIIL 158 Query: 475 RF-----EDNKEKK-----CQMKTAGQIQDISVETDALLLWVNVHNFSAKY- 597 RF EDNKEKK CQMKTAG Sbjct: 159 RFQIQDISVETEDNKEKKSAKDALLLWCQMKTAGYPN-------VNVHNFTTSWR 206 Query: 598 PNLAFNAIVHKHRPDLLDTTSWRDGFESLKKCNAHYNLQNAFNLAEKELGLTKLLDP--- 768 LAFNAIVHKHRPDLLD FESLKKCNAHYNLQNAFNLAEKELGLTKLLDP Sbjct: 207 DGLAFNAIVHKHRPDLLD-----FESLKKCNAHYNLQNAFNLAEKELGLTKLLDPEDV 259 Query: 769 NVDQPDEKSIITYKMKALAVEDVVSTYYHYFS-----RIGKVLDHAMEAEASELL 918 NVDQPDEKSIITY V+TYYHYFS RIGKVLDHAMEAE Sbjct: 260 NVDQPDEKSIITY------VATYYHYFSKMKALAVEGKRIGKVLDHAMEAE---- 304 Query: 919 QWEGKHLVEKYESL-----TIGTFNDRQLANS---VQNQLQSFNSYRTIEQLSGV 1059 HLVEKYESL TI T NDRQLANS VQNQLQSFNSYRT V Sbjct: 305 ----HLVEKYESLASELLQWIEQTIVTLNDRQLANSLSGVQNQLQSFNSYRT----- 353 Query: 1060 EKPPKFTEKGNLEVL---IQSKLRANNQKVY---EGRLILFTTPRSDINKAWERLEKAEH 1221 EKPPKFTEKGNLEVL IQSKLRANNQKVY EGRLI SDINKAWERLEKAEH Sbjct: 354 EKPPKFTEKGNLEVLLFTIQSKLRANNQKVYTPREGRLI-----SDINKAWERLEKAEH 407 Query: 1222 ERELALRTEAARFDRENQRLVLIRQEKLEQL-----RETWLS-----FGL 1341 ERELALRTE LIRQEKLEQL RETWLS Sbjct: 408 ERELALRTE-----LIRQEKLEQLAARFDRKAAMRETWLSENQRLVSQDNFGL 455 Query: 1342 ELAKAAMSQDNRKHEAIETDIVAYSGRXXXXXXXXXXXXXXXXXXXXHYHDIKRIAARQN 1521 EL AA+ RKHEAIETDIVAYSGR HYHDIKRIAARQN Sbjct: 456 EL--AAVEAAVRKHEAIETDIVAYSGR-----VQAVDAVAAELAAEHYHDIKRIAARQN 507 Query: 1522 NVARLWDFLREMVAAXXXXXXXXXXXQKVFQDLLYLMQSQDLGQLHELVDWMAEMKGRL- 1698 NVARLWDFLREMVAA QKVFQDLLYLM Sbjct: 508 NVARLWDFLREMVAARRERLLLNLELQKVFQDLLYLM------DWMAEMKGRLQ 555

Query:	1699	KHLAGVEDLLEYIAVQAERVRAVSAYRPCGEQSYESALRFCDPGKEXX	1842
Sbjct:	556	KHLAGVEDLL+ IAVQAERVRAVSA SALRFCDPGKE SQDLGKHLAGVEDLLQLHELVEADIAVQAERVRAVSASALRFCDPGKEYR	605
Query:	1843	XXXXXXXXXPQLVSALCELRRLWRFLWEVGEAEAWVREQQHLLA Q ALCEL RRLWRFLWEVGEAEAWVREQQHLLA	1977
Sbjct:	606	PCDPQLVSERVATLEQSYEALCELAATRRARLEESRRLWRFLWEVGEAEAWVREQQHLLA	665
Query:	1978	SAETGRDLTGVLRLLNKHTALRGLTLEQGNQASTEMSGRLGPLKQQLVAEGHPG SAETGRDLTGVLRLLNKHTALRG EMSGRLGPLK QQLVAEGHPG	2139
Sbjct:	666	SAETGRDLTGVLRLLNKHTALRGEMSGRLGPLKLTLEQGQQLVAEGHPG	714
Query:	2140	ALQXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	2286
Sbjct:	715	ANQASTRAAELQAQWERLEALAEERAQRLAQAASLYQFQADANDMEAWLVDALR	768
Query:	2287	LVSSPEVGHDEFSTQARALEEEIXXXXXXXXXXXXQHPTLDALREQAA LVSSPEVGHDEFSTQA RALEEEI PTLDALREQAA	2430
Sbjct:	769	LVSSPEVGHDEFSTQALARQHRALEEEIRAHRPTLDALREQAAALPPALS	818
•		EVQGRVPTLEQRARALLSHTPHYEELQARAGEFYTMLSEPGACNXXX EVQGRVPTLEQ HYEELQARAGE FYTMLSE GAC	
_		HTPEVQGRVPTLEQHYEELQARAGERARALEAALAFYTMLSEAGACG	
Query:	2572	XXXXXXXWVEEKEQWLDPEVVQQRFETITAVSPERLELEPEMNALAAR-WVEEKEQWL D EVVQQRFET LEPEMNALAAR	2718
Sbjct:	866	LWVEEKEQWLNGLALPERLEDLEVVQQRFETLEPEMNALAARV	908
Query:	2719	DIAEQLLKASPPGKDRIIGTQEQLNQRWQQFRSLAGGKKAALTSALSI	2862
Sbjct:	909	TAVSDIAEQLLKASPPGKDRIIGTQEQLNQRWQQFRSLADGKKAALTSALSIQNYHLECT	968
Query:	2863	ETQAWMREQDLGNDLAQNYHLECTKTKVIESTGVLALQRKLAGTERDL*ADP ETQAWMRE QDLGNDLA GVLALQRKLAGTERDL A	
Sbjct:		ETQAWMREKTKVIESTQDLGNDLAGVLALQRKLAGTERDLEA-I	
•		RAK*SHLCPGGFGCWAPSPSPCHQHTMGGSSGNPGLARRLGEVQTMRRREES A+ L A P+ P + RLGEVQ TMRRREES	
		SARVGELTQEANALAAGHPAQAPAINTRLGEVQTGWEDLRATMRRREES	1060
Query:	3175	DFLRSLDDFQAWLGRRLQTQTAVASYE 3255 DFLRSLDDFQAWLGR TQTAVAS E	
Sbjct:	1061	LGEARRLQDFLRSLDDFQAWLGRTQTAVASEE 1092	
	lties	40 bits (1910), Expect(3) = 0.0 = 536/931 (57%), Positives = 545/931 (58%), Gaps = 144/931 (1	<u>.</u> 5%)
		QAWMGEQEKAKDELSAQVLEQALALHMMGQEQAEVKKHDYAQTIKQ	
Sbjct:	1,601	+AWMGEQE KAKDELSAQ VLEQALA DYAQTIKQ EAWMGEQELHMMGQEKAKDELSAQAEVKKHQVLEQALADYAQTIKQ	1646
Query:		LAASSQDMIDHEHPESTRLTIRGLKELAQAQVDKLYARERLQEHLRLCQXX	5065
Sbjct:		LAASSQDMIDHEHPESTRLTIR QAQVDKLYA RERLQEHLRLCQ LAASSQDMIDHEHPESTRLTIRQAQVDKLYAGLKELAGERRERLQEHLRLCQLR	1700
Query:	5066	XXXXXXXXXXXXXSHELGQDYEHEQWIQERVTMLRDKFREFSRDTDSXXXXXXXX	5230



results of NLASI

100.00

97.06

30527 gi|3452552|dbj|AB001347.1|

1 30527 gi|3452552|dbj|AB001347.1|

1 30527	gi 3452552 db	i AB001347.1	100.00	31	0	0	2080		2110
	gi 3452552 db		100.00	30	0	0	6494		6523
_	gi 3452552 db		100.00	30	0	0	3873		3902
	•	-			-				
_	gi 3452552 db		100.00	30	0	0	1699		1728
	gi 3452552 db		100.00	30	0	0	1285		1314
	gi 3452552 db		96.88	32	1	0	5229		5260
1_30527	gi 3452552 db	j AB001347.1	100.00	29	0	0	523		551
1 30527	gi 3452552 db	j AB001347.1	100.00	28	0	0	3606		3633
1 30527	gi 3550974 db	AB008551.1	92.00	300	0	12	193		480
1 30527	gi 3550974 db	i IAB008551.1	81.78	516	40	43	3633		4115
	gi 3550974 db	•	86.09	381	9	24	4173		4530
_	gi 3550974 db		84.07	427	9	24	4142		4530
_	-	-		193	1	0	1440		
_	gi 3550974 db		99.48			_			1632
	gi 3550974 db		100.00	144	0	0	1903		2046
	gi 3550974 db		98.65	148	2	0	2719		2866
_	gi 3550974 db	-	95.92	147	0	2	5285		5431
	gi 3550974 db		100.00	121	О .	0	7091		7211
1_30527	gi 3550974 db	j AB008551.1	93.75	144	0	2	673		807
1 30527	gi 3550974 db	j AB008551.1	93.15	146	1	1	3398		3543
1 30527	gi 3550974 db	j AB008551.1	86.87	198	7	11	2686		2866
1 30527	gi 3550974 db	AB008551.1	93.33	135	0	6	6551		6676
_	gi 3550974 db		100.00	91	0	0	4888		4978
_	gi 3550974 db		98.89	90	1	0	6146		6235
_	gi 3550974 db		98.90	91	0	1	4709		4798
_	gi 3550974 db			136		7			
	•		86.76		0		5624		5741
	gi 3550974 db		81.54	195	8	19	5561		5741
_	gi 3550974 db	•	98.70	77	0	1	1176		1252
_	gi 3550974 db		91.09	101	0	4	5957		6057
	gi 3550974 db		84.06	138	12	10	6865		6997
1_30527	gi 3550974 db	j AB008551.1	90.62	96	0	1	1057		1143
1_30527	gi 3550974 db	j AB008551.1	84.25	127	5	9	5024		5135
1 30527	gi 3550974 db	[AB008551.1]	87.50	96	0	4	2143		2238
1 30527	gi 3550974 db	j AB008551.1	98.18	55	1	0	6406		6460
_	gi 3550974 db	•	88.10	84	4	5	4610		4688
	gi 3550974 db		91.55	71	3	3	2937		3006
_	gi 3550974 db		82.35	119	10	10	6281		6392
	gi 3550974 db		83.19	113	6	9	5453		5560
_	gi 3550974 db			49	0	0			
			100.00				1375		1423
	gi 3550974 db		87.36	87	2	2,	961		1038
	gi 3550974 db		100.00	48	0	0	604		651
	gi 3550974 db		100.00	48	0	0	1		48
	gi 3550974 db		100.00	47	0	0	3050		3096
_	gi 3550974 db	•	100.00	45	0	0	6731	1	6775
	gi 3550974 db		100.00	45	0	0	5831		5875
1_30527	gi 3550974 db	j AB008551.1	100.00	45	0 .	0	5156		5200
1_30527	gi 3550974 db	AB008551.1	84.38	96	0	8	2287	:	2367
1 30527	gi 3550974 db	AB008551.1	100.00	42	0	0	1733		1774
_	gi 3550974 db		100.00	39	0	0	865	9	903
	gi 3550974 db		100.00	36	0	0	6074		6109
	gi 3550974 db		100.00	36	0	0	3291		3326
_	gi 3550974 db		100.00	36	0 .	0	133		3320 168
_	gi 3550974 db		100.00	34	0	0	6923		6956
	gi 3550974 db		100.00						
				34	0	0	2431		2464
_	gi 3550974 db		100.00	34	0	0	2398		2431
_	gi 3550974 db		100.00	33	0	0	2590		2622
	gi 3550974 db		100.00	33	0	0	2494		2526
	gi 3550974 db		97.22	36	1	0	2256		2291
	gi 3550974 db		100.00	33	0	0	2110		2142
	gi 3550974 db		100.00	32	0	0	3961		3992
1_30527	gi 3550974 dbj	j AB008551.1	100.00	32	0	0	1667		1698

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        AUG 18
                Data available for download as a PDF in RDISCLOSURE
NEWS
     6
NEWS
     7
        AUG 18
                Simultaneous left and right truncation added to PASCAL
NEWS 8
        AUG 18
                FROSTI and KOSMET enhanced with Simultaneous Left and Righ
                 Truncation
        AUG 18
                Simultaneous left and right truncation added to ANABSTR
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NEWS 10
        SEP 22
NEWS 11 SEP 25
                INPADOC: Legal Status data to be reloaded
                DISSABS now available on STN
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NEWS 13 OCT 10
                PCTFULL: Two new display fields added
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                BIOSIS file reloaded and enhanced
                BIOSIS file segment of TOXCENTER reloaded and enhanced
NEWS 15 OCT 28
NEWS EXPRESS
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=> S SPECTRIN AND RATTUS

L2 2 SPECTRIN AND RATTUS

=> DUP REM L2

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- AN 2002:161955 BIOSIS
- DN PREV200200161955
- TI Synaptic scaffolding proteins in rat brain. Ankyrin repeats of the multidomain Shank protein family interact with the cytoskeletal protein alpha-fodrin.
- AU Boeckers, Tobias M.; Mameza, Marie Germaine; Kreutz, Michael R.; Bockmann, Juergen; Weise, Christoph; Buck, Fritz; Richter, Dietmar [Reprint author]; Gundelfinger, Eckart D. [Reprint author]; Kreienkamp, Hans-Juergen
- CS Institut fuer Zellbiochemie und klinische Neurobiologie, Universitaet Hamburg, Martinistrasse 52, 20246, Hamburg, Germany richter@uke.uni-hamburg.de; gundelfinger@ifn-magdeburg.de
- SO Journal of Biological Chemistry, (October 26, 2001) Vol. 276, No. 43, pp. 40104-40112. print. CODEN: JBCHA3. ISSN: 0021-9258.
- DT Article
- LA English
- ED Entered STN: 21 Feb 2002

Last Updated on STN: 26 Feb 2002

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- L3 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
- AN 1998:472526 BIOSIS
- DN PREV199800472526
- TI Ancient large-scale genome duplications: Phylogenetic and linkage analyses shed light on chordate genome evolution.
- AU Pebusque, Marie-Josephe; Coulier, Francois; Birnbaum, Daniel [Reprint author]; Pontarotti, Pierre

CS Inst Cancerologie d'Immunologie de Marseille, Univ. Mediterranee, INSERM U 119, 27 Bd Lei Roure 13009 Marseille, France

SO Molecular Biology and Evolution, (Sept., 1998) Vol. 15, No. 9, pp. 1145-1159. print.

CODEN: MBEVEO. ISSN: 0737-4038.

DT Article

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ED Entered STN: 5 Nov 1998

Last Updated on STN: 5 Nov 1998

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=> S GTRAP* AND RATTUS

L5 0 GTRAP* AND RATTUS

=> S SPNB3 AND RATTUS

L6 0 SPNB3 AND RATTUS

=> S SPN* AND RATTUS

L7 0 SPN* AND RATTUS

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